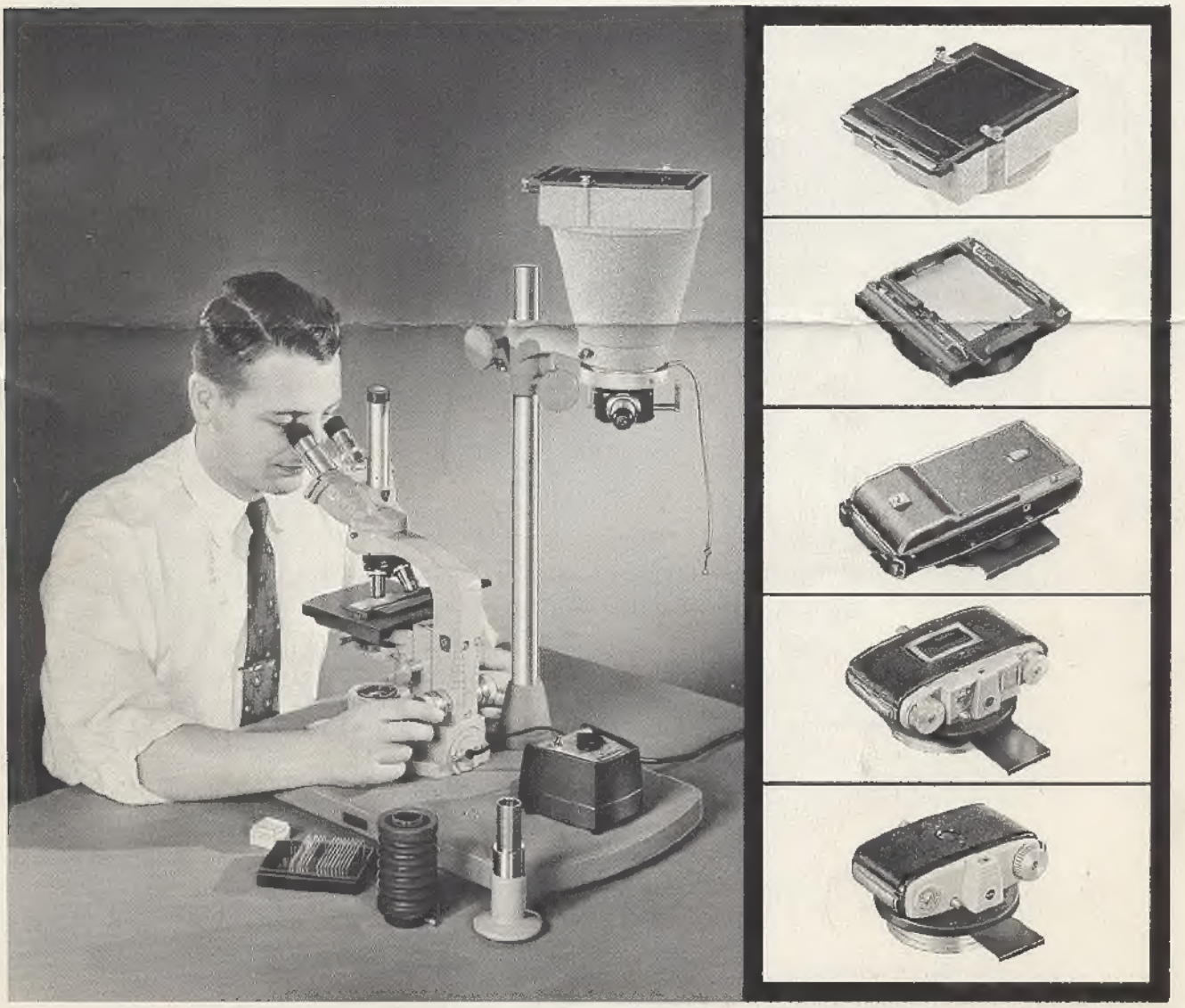
SPENCER

SERIES 682 PHOTOMICROGRAPHIC CAMERAS

REFERENCE MANUAL



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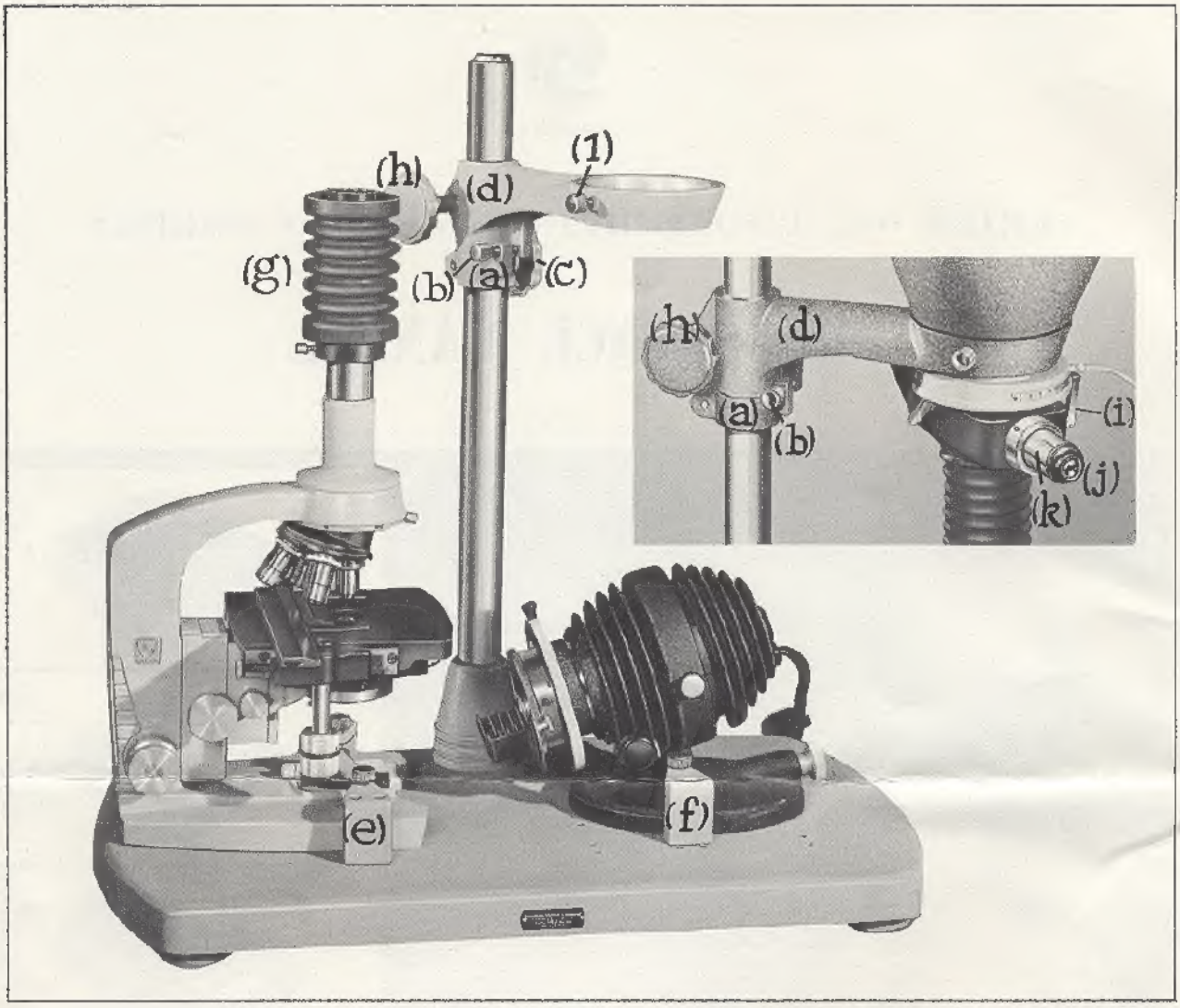


Figure 1. AO Spencer No. 682 Camera with Microstar Microscope and No. 735 Advanced Laboratory Microscope Illuminator.

(a) Lower Assembly

(b) Fine Adjustment Screw

(c) Lock Knob

(d) Upper Assembly

(e) Clamp Assembly

(f) Clamp Assembly

(g) Light-Tight Connector

(h) Lock Knob

(i) Shutter Trip Lever

(j) Wide Field Eyepiece

(k) Telescopic Tube

(l) Lock Screw

INTRODUCTION

Successful photomicrography is basically dependent upon: an efficient, controllable source of illumination; proper use of a good microscope; correct alignment of illuminator to microscope; and precise alignment of the photomicrographic camera to the optical and geometrical axis of the microscope and illuminator.

The prime purpose of this reference manual is to give you a step-by-step procedure to follow in order to effectively set up and use your Series 682 Camera and accessories to full advantage. Supplementary notes and an abbreviated list of references are also included for guidance.

A. ASSEMBLY OF CAMERA AND ALIGNMENT TO MICROSCOPE

1. Insert lower assembly (a) onto vertical column...set "fine" adjustment screw (b) at mid-travel...and lock assembly approximately six inches from top of column with lock knob (c).

2. Insert upper assembly (d) onto vertical column and assembly (a).

3. Attach cable release...set shutter dial to "time" (T) position...and open shutter. Do not assemble camera back into camera support arm (d) until step B.

4. Place microscope on baseplate as illustrated in figure (1) so that microscope base fits equally between and freely under clamp assemblies (e).

5. Release lock knob (c)...simultaneously swing camera back support arm (d) and assembly (a) clockwise...and at the same time raise or lower the combined assemblies...until, while peering vertically through top of the assemblies, the microscope monocular body is approximately 1" below and directly concentric to the underneath aperture of assembly (b). Lock knob (c).

   It may be necessary to reposition your microscope and readjust the relative position of the swinging arm assemblies to satisfy the above conditions. Lock clamps (e). Swing support arm (d) counterclockwise from optical axis.

6. If your microscope is equipped with a built-in base or other integral illuminator, skip step 7 and proceed to step 8.

7. Place illuminator on baseplate as illustrated in figure (1)...direct light beam at proper incidence to microscope mirror.

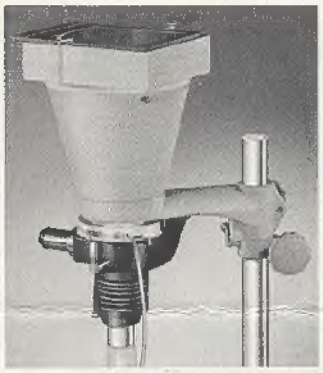
8. Place eyepiece into microscope body tube...focus onto suitable specimen with 10X objective...observe standard practice for field and aperture diaphragm settings...precisely align illumination to the microscope. Lock clamps (f).

9. Insert flexible light-tight connector (g) over monocular body...compress flexible bellows...swing camera support arm (d) clockwise to fine adjustment stop screw (a)...release and seat top adapter into aperture... and lock knob (h).

10. Close shutter...raise mirror lever located opposite shutter trip lever (i).

11. Focus Wide Field eyepiece (j) onto cross-hair reticle, contained in telescopic tube (k), by sliding eyepiece in or out. Refine coincidence of field, if necessary, by releasing lock knob (h) and adjusting fine adjustment (b); and / or slightly repositioning microscope. Secure all clamps and locking screws.

Your photomicrographic outfit is now ready for use. Above steps need not be repeated unless alignments have been disturbed.



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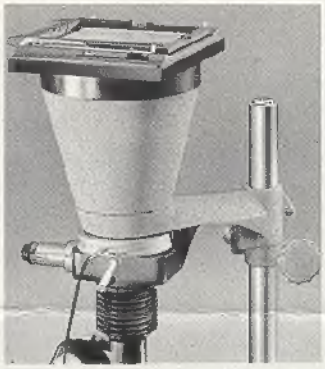

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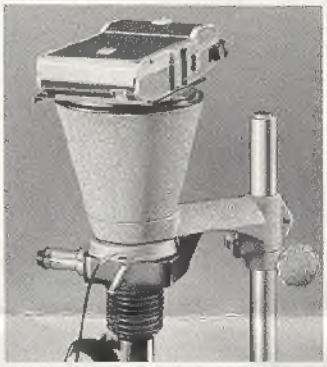

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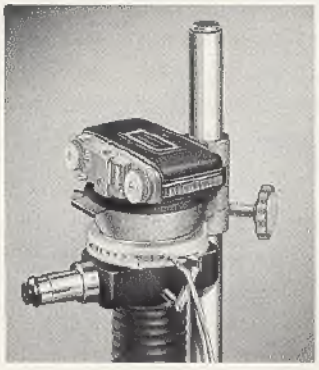
657 Adapter for 687, 688,

Figure 2. Wide selection of AO Photomicrographic Camera Backs. All are interchangeable...rotatable.



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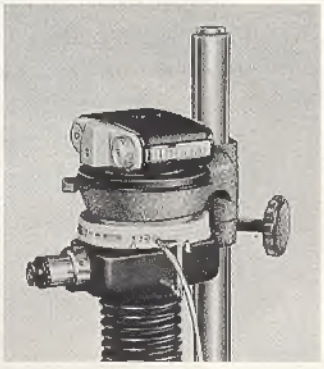

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B. PROCEDURE FOR PHOTOMICROGRAPHY

Assuming that microscope, camera and illuminator have been properly aligned in accordance with Section A:

1. Place specimen slide on stage...select objective, eyepiece and filters to be used...focus visually and correctly...adjust condenser height, field and aperture diaphragms.

2. Insert light-tight connector over monocular body...swing camera support (d) clockwise to definite stop and lock knob (h). Use of light-tight connector (g) is not absolutely necessary.

3. Loosen lock screw (l)...insert cone-shaped adapter and desired preloaded camera back into camera support (d) and tighten lock screw (l). 35mm camera backs engage directly into (d). All backs are freely interchangeable and rotatable for specimen orientation. (See Figure 2)

4. Raise mirror lever located opposite shutter trip lever (i) to direct image through telescopic tube (k).

5. View through camera eyepiece (j) focus eyepiece within slotted telescopic tube (k) until cross-hair reticle within the latter is in sharp focus. Rotate microscope fine adjustment so that specimen detail is in sharp focus coincident with cross-hair.

6. Set shutter at selected exposure time...draw out dark slide from film holder.

7. Recheck focus...allow slack in cable and trip self-cocking shutter... insert dark slide. If rollfilm is used, immediately wind film to next frame to avoid double exposure.

8. Develop black and white or color film in accordance with manufacturer's recommendations or have film processed by a competent and reputable processor.

C. DETERMINATION OF EXPOSURE TIME

The determination of correct exposure time is dependent upon such factors as type of film, development, light intensity and color temperature, magnification, aperture of objective, density of specimen and filters.

Generally, a series of test exposures should be used to establish significant constants. Black and white film such as Panatomic X ASA-20 is suggested for test runs because of economy and time.

1. For 35mm film, take several frames at various unit exposures...select the best after development. For sheetfilm, Polaroid, and plates, pull out the dark slide in increments of approximately one inch...expose after each move until entire sheet or plate is covered...develop...select the best.

2. Carefully record all conditions, such as: exposure time, eyepiece, objective, light source, filters, diaphragm settings, film and developer.

3. Once an acceptable exposure time has been established, by the above method, other correct exposure time for differences of film, filters, magnification can easily be computed; or, determined by use of a suitable light meter such as Photovolt Exposure Photometer AO #1342. The photocell unit may be positioned onto camera eyepiece (j).

Thickness of a specimen has little or no influence on exposure time. When a photometer is used - always focus onto the specimen...move slide to clear area for uninterupted light measurement...and record exposure time value of this area.

EXPOSURE GUIDE

The following exposures generally apply for average density stained slides when using AO Model 735 Illuminator without filters and adjusted for Koehler illumination; Ektachrome X and Panatomic F film; 10X eyepiece; top lens element of Abbe condenser removed for use with 5X and 10X objectives; Photovolt Meter (Model 200M) positioned at telescopic eyepiece:

Table I. Exposure Guide for Camera Backs 687, 688 and Reduced Cutfilm

Objective Magnification	Photovolt Reading Model 200 M	Exposure Time	
		Ekta F ASA-12	Pana X ASA-20
5X	330	1/2 second	3/10 second
10X	330	1/2	3/10
20X	330	1/2	3/10
43X	100	2	1
97X	45	4	2

Table II. Exposure Guide for Camera Backs 668, 669

Objective Magnification	Photovolt Reading Model 200 M	Exposure Time	
		Ekta F ASA-12	Pana X ASA-20
5X	330	1/10 second	1/25 second
10X	330	1/10	1/25
20X	330	1/10	1/25
43X	100	1/2	1/10
47X	45	1	1/5

Note that the required exposure times for photomicrography with Camera Back #668 35mm and #669 Bantam (828 Rollfilm) are approximately 1/5 that required for Camera Back 687 4" x 5" and #688 4" x 5" Graflok.

D. SUPPLEMENTARY NOTES

1. FILTERS

a. For Black and white photomicrography - Wratten 58 is usually preferred. Exposure time must be extended proportionally (e.g.) increase exposures 5 times when Wratten 58 is used with Panatomic X.

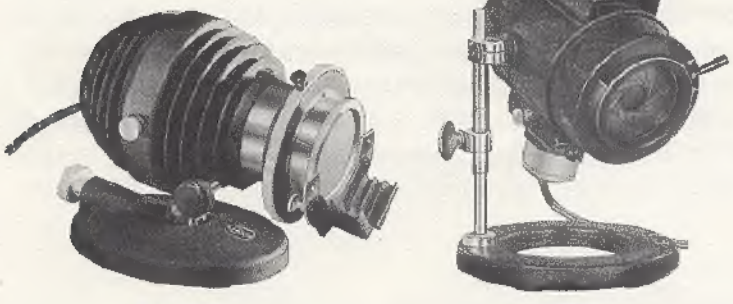
b. For Color Photomicrography -

Film manufacturers suggest the use of color compensating filters, such as, EK82A and / or 82C to raise color temperature to 3800 Kelvin which is the correct temperature for Kodachrome F, Ektachrome F and Anscochrome Flash. Some users prefer 82C only; others use 82A and 82C together; many simply use only the small blue glass furnished with microscopes. In short, it's a matter of personal choice.

If you elect to use these color compensating filters, extend exposure 1/2 time for either filter. EK Co. suggests that you use compensating filters sparingly, if at all, with Ektachrome F when exposure times greater than 1/2 seconds are required because of film reciprocity effects. AO #607 Didymium filter (41mm diameter), or #608 (33mm diameter), 2-1/2mm thick are highly recommended for H&E stained or predominantly red specimens to enhance red and suppress yellow...extend exposure 1/2 time.

Wratten and color compensating filters are available in various sizes from larger photo outlets and laboratory supply houses... not from American Optical Company.

2. ILLUMINATION



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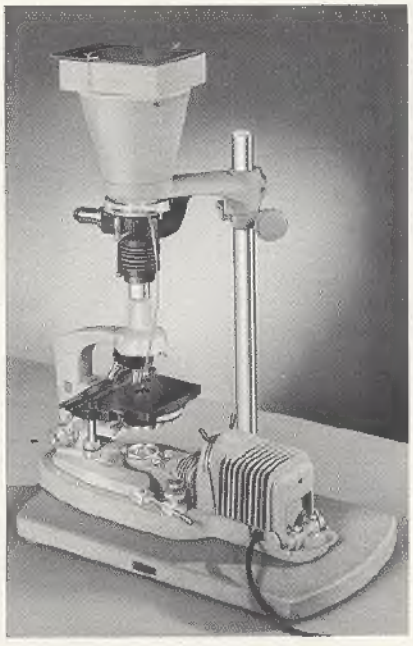

Figure 3. Above: No. 735C Advanced Laboratory Microscope Illuminator; No. 370 Adjustable Laboratory Microscope Illuminator. At right: No. 600 Ortho-Illuminator.

Light intensity is of prime importance so far as exposure factors are concerned. Intensity of illumination should be modified by insertion of neutral density filters, such as AO #743, 744, 745...not by reduction of voltage, improper focusing of condenser, or closure of condenser aperture diaphragm.

The rated color temperature of various bulbs is directly dependent upon applied voltage. If line voltage is lower than bulb rating, the light appears yellow...if higher, it appears whiter. Raising voltage with a variac or transformer may help at the sacrifice of bulb life...so, generally, color compensation filters mentioned in section D1b are recommended for this purpose.

A simple 0-10 voltmeter (Triplex-tradename), obtainable from electronic supply houses can be attached to transformers of low voltage illuminators to permit exact match points for repetitive exposures and enable user to offset line fluctuations.

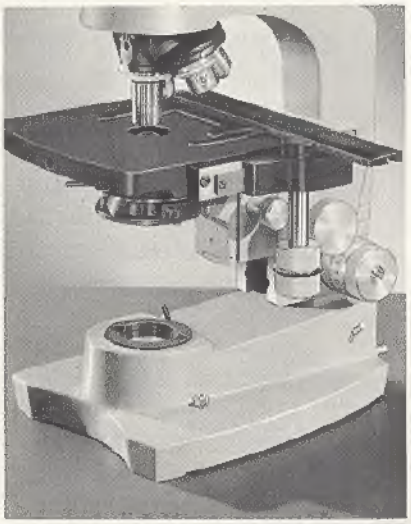

Figure 4. Built-in Base Illuminator on Microstar Series 4.

If AO MICROSTAR Series 4 microscope (equipped with built-in base illuminator) is used, set transformer at 7-1/2 volts and extend exposure 5 times that shown in Tables I and II.

3. FILM RATING

American Standards Association (ASA) ratings designate the speeds of various films and photographic plates. If you find it desirable to use a faster or slower film, compute revised exposure time by the relative ASA ratings: (e.g.) a film of ASA-25 Tungsten requires approximately four times the exposure of an ASA-100 Tungsten; Kodachrome A is about eight times slower, etc.

4. MAGNIFICATION

The resultant magnification at the plane of the photographic film or plate of No. 687 4" x 5" fixed camera back, No. 688 4" x 5" Graflok camera back and No. 689 Polaroid Land Camera is equal to the product of the initial magnification of the objective and magnification of the eyepiece. For example: a 10X objective used in combination with a 10X compensating eyepiece produces a resultant magnification of 100X.

The resultant magnification at the film plane of No. 668 35mm camera back and No. 669 Bantam Camera Back is equal to 40% of the product of the objective and eyepiece powers. For example: Resultant magnification = .40 x 10 x 10 = 40X.

The resultant magnification obtained through focusing telescopic system (j, k) is equal to ten times the product of the objective and microscope eyepiece and is very desirable for critical focus. Since field of view is proportional to magnification, this resultant magnification of 1000X (for above combination) produces a smaller field of view than that actually projected toward the photographic film or plate.

E. SUBSTAGE CONDENSER

Focus your microscope condenser as critically as possible so that iris diaphragm of the illuminator is simultaneously in sharp focus with the specimen image. If the condenser is incorrectly focused too high or too low a predominance of blue or red, respectively will detract from the fidelity of color shots.

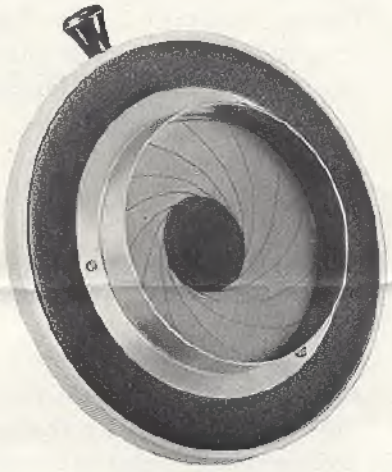

Figure 5. Field iris diaphragm of No. 735 Illuminator.

Use 10X 16mm Objective...focus sharply on specimen...close field iris diaphragm of illuminator until it appears just within the field of view...adjust condenser height until the closed iris leaves of the illuminator are sharply in focus at the same plane as the specimen...open the field diaphragm so that it just clears the field of view. This condenser height setting need not be changed for all other objectives or specimen slides of same thickness. The field diaphragm, however, must be readjusted to just clear the field-of-view for each respective objective when specimen is viewed through the microscope eyepiece.

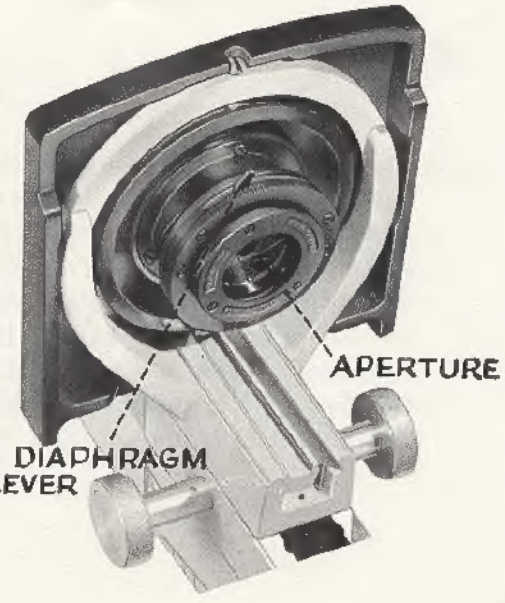

Figure 6. Aperture iris diaphragm of substage condenser.

The aperture iris diaphragm of the condenser -- not to be confused with the field diaphragm of the illuminator -- controls the numerical aperture of the illuminating cone emerging from the condenser and must also be properly adjusted for each objective. Having properly focused onto the specimen and correctly adjusted the condenser height and field diaphragm: remove the eyepiece...view down the microscope tube with your eye at approximately reading distance...observe the image of the condenser aperture diaphragm in the rear lens of the objective... close or open this iris diaphragm until it is coincident with the full area or aperture of the rear objective lens. If you experience difficulty for the proper setting of the aperture diaphragm to higher magnification objectives, a telescopic eyepiece, pinhole eyepiece or Bertrand lens should be used.

In most cases the aperture diaphragm should be closed to about 2/3 to 4/5 of total aperture if enhanced contrast and increased depth of focus is desired. It should, however, never be closed to the extent that diffraction patterns falsify the microscopic image...nor should it be used to control light intensity.

F. CURVATURE OF FIELD

Curvature of field is prevalent in all objectives...paradoxically more so in apochromats than achromats because of higher numerical apertures.

The best way to reduce curvature is to focus off dead center and approximately one-third the distance from center to the periphery of the field. Closing the aperture iris diaphragm of the condenser, without excessively encroaching on numerical aperture, will also enhance results. Compensating eyepiece 10X must be used with apochromatic objectives and is frequently preferred for photomicrography with achromatic objectives.

G. 2¼ x 3¼ CUT FILM

The most economical size for projection of 3-1/4 x 4 slides is 2-1/4 x 3-1/4 cut film. Lower cost - no waste. Use as follows: (1) Place #2420B plate holder kit in #670 plate holder. (2) Use 2424 film sheath and space so that 2-1/4 x 3-1/4 film placed in short dimension is centered to the long dimension. (3) Cut piece of black cardboard 3-1/4" long and 1" wide. (4) Slide this piece in #2424. (5) This will center cut film 2-1/4 x 3-1/4 in a 3-1/4 x 4-1/4 sheath (#2424). (6) Suggest marking ground glass 2-1/4 x 3-1/4 to reference coverage of projected image. (See Figure 7)

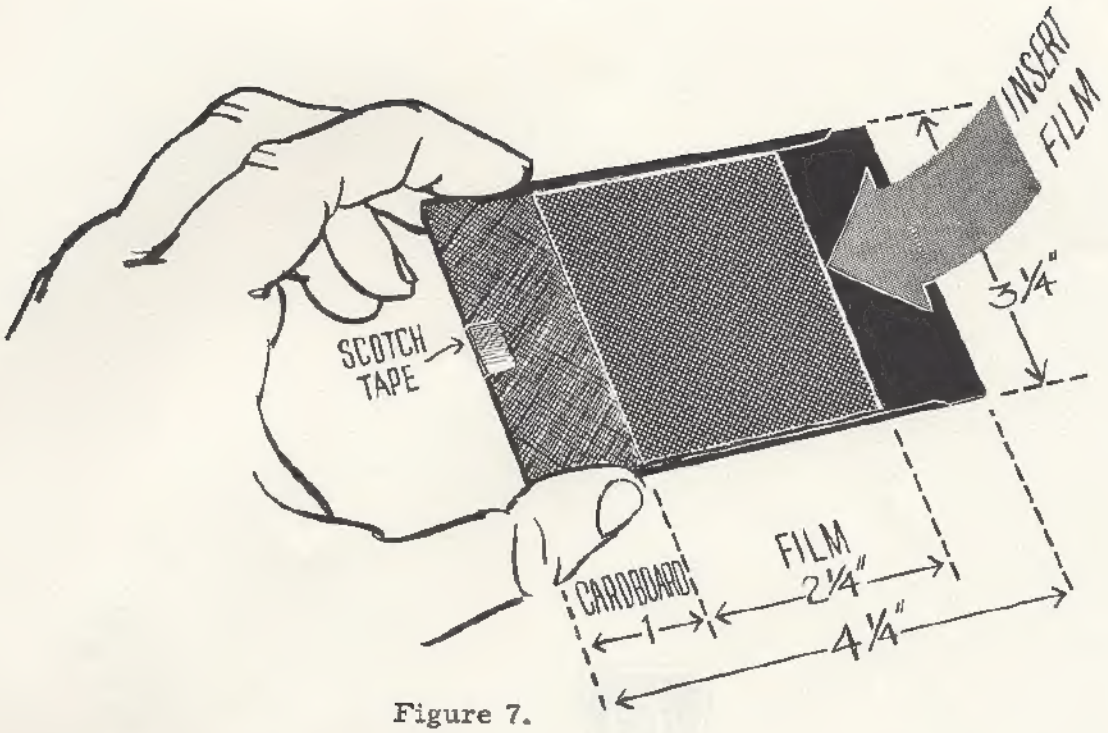

Figure 7.

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